

Certificate of Analysis for NR-52390

Adenovirus Serotype 5, Clone Ad5-CMV-hACE2/RSV-eGFP, Recombinant Expressing Human ACE2

Catalog No. NR-52390

Product Description:

Human angiotensin-converting enzyme 2 (ACE2; GenBank: AB046569) complementary DNA was cloned into E1/E3-deleted recombinant adenovirus serotype 5 (Ad5), driven by a cytomegalovirus (CMV) promoter, along with an enhanced green fluorescent protein (GFP) gene, driven by a respiratory syncytial virus (RSV) promoter. NR-52390 is an adenoviral vector engineered to express human ACE2, the receptor of severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2). NR-52390 lot 70035407 was produced by infecting human embryonic kidney cells (HEK-293; ATCC® CRL-1573™) with the deposited material and incubating in Dulbecco's Modified Eagle's Medium (ATCC® 30-2002™) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 2 days at 37°C with 5% CO₂.

Passage History:

Unknown/HEK-293(1) (prior to BEI Resources/BEI Resources)

Lot: 70035407 Manufacturing Date: 02MAY2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in HEK-293 Cells	Cell rounding and sloughing GFP expression	Cell rounding and sloughing GFP expression (Figure 1)
Genotypic Analysis by Next-Generation Sequencing	ACE2 sequence confirmed eGFP sequence confirmed	ACE2 sequence confirmed eGFP sequence confirmed
Titer by TCID₅₀ Assay in HEK-293 Cells by Cytopathic Effect and GFP Readout¹ (7 days at 37°C and 5% CO₂)	Report results	1.6 × 10 ⁹ TCID ₅₀ per mL
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic ²	No growth	No growth
Trypticase Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C and 5% CO ₂	No growth	No growth
Mycoplasma Contamination		
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

¹The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation. ²Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

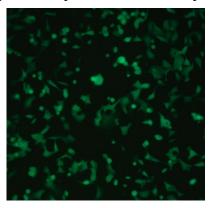
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Identification by Infectivity in HEK-293 Cells by GFP Expression



/Heather Couch/ <u>Heather Couch</u>

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Program Manager or designee, ATCC Federal Solutions

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